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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 14/52, A61K 38/19, C07K 16/24

A1

(11) International Publication Number: WO 98/51705

(43) International Publication Date: 19 November 1998 (19.11.98)

(21) International Application Number: PCT/EP98/02750

(22) International Filing Date: 11 May 1998 (11.05.98)

(30) Priority Data:
MI97A001105 12 May 1997 (12.05.97) IT

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PEPTIDES WITH ANTIVIRAL ACTIVITY

(57) Abstract

There are disclosed peptides with a sequence corresponding to the proteic domain comprised between the second and the third cysteine of chemokines of the CC family, particularly RANTES, MIP–1α and MIP–1β and the derivatives thereof. The peptides of the invention are useful for the treatment of diseases caused by the infection of viruses like HIV–1, other primate lentiretroviruses (HIV–2, SIV) and other viruses which use chemokine receptors to bind the cellular surface and/or to penetrate the target cell, or they can be used for the treatment of allergic or autoimmune diseases, in the pathogenesis of which the chemokines play an important role.

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PEPTIDES WITH ANTIVIRAL ACTIVITY

The object of the present invention is to provide peptides with a sequence corresponding to the protein domain comprised between the second and third cysteine of chemokines of the C-C family, particularly of RANTES, MIP-1α and MIP-1β, and the derivatives thereof.

The peptides of the invention are useful for the treatment of diseases which are connected with the infection of viruses like HIV-1, other primate-lentiretroviruses (HIV-2, SIV) and other viruses which use chemokine receptors to bind the cellular surface and/or to penetrate the target cell, as well as for the treatment of all the diseases, like the allergic or autoimmune diseases, in the pathogenesis of which chemokines play an important role.

Background of the invention

The term chemokine is used to identify a family of chemotactic cytokines characterized by a high degree of genetic, structural and functional similarity (Immunol. Today 1993, 14:24).

Most of the known chemokines are grouped in two main families referred to as C-X-C and C-C, depending on the configuration of a conserved motif of two cysteine in their sequence (Ann. Rev. Immunol. 1994, 55:97-179).

Chemokines are important mediators of the inflammatory response which act through the recruitment of specific cellular populations of the immune system in the inflammatory site; the C-X-C chemokines are generally active on neutrophil granulocytes while the C-C chemokines are active on eosinophil and basophil

granulocytes, on limphocytes and monocytes.

RANTES, MIP-1α and MIP-1β are C-C chemokines which have been proposed as possible mediators of autoimmune and allergic diseases.

Recently, a specific antiviral effect against primate lentiretrovirus has been described for those three chemokines (Science, 1995, 270:1811-1815).

The observed antiviral activity has been attributed to the capability of some chemokines (like RANTES, MIP-1α and MIP-1β) to interact with the CCR5 co-receptor of HIV-1 (Cell, 1996, 85:1135-1148; Science 1996 272:1955-1956; Nature, 1996, 381:651-666; ibidem, 667-678; Cell, 1996, 85:1149-1158). Another chemokine of the C-X-C family, named SDF-1, interacts with the $CXCR_4$ receptor, or fusin, which is used as co-receptor by those HIV-1 and HIV-2 strains which grow in continuous cell lines. Thus SDF-1 interrupts the growth of such strains which are present in a high percentage of patients (higher than 50%) after the onset of clinical symptoms of HIV disease.

However, the therapeutic use of natural chemokines is hampered by their pro-inflammatory activity which is mainly caused by the induction of chemotaxis (i.e. the directional movement of the cell, which is determined by a chemical gradient, and the consequent local accumulation of the responsive cells), through the binding and the activation of the corresponding receptor on the cellular surface, by the functional activation of the cell (for instance the release of istamine by basophyl granulocytes) and by the induction of cellular proliferation through the secretion of growth factors (for

instance the activation of the cell cycle and the IL-2 release from T-limphocytes). At the moment the pro-inflammatory activity represents one of the main bar to the use of wild-type chemokines in the therapy of HIV infection. In fact, the doses necessary for obtaining a therapeutic effect could cause toxic effects due to the generation of inflammatory processes diffused or localized in the organs where the drug mostly accumulates (e.g. in the kidney, thus causing glomerulonephritis, or in the liver, thus causing epatitis, etc.).

At the moment a preliminary knowledge exists of the domains involved in the pro-inflammatory activity of some chemokines, but not of the domains involved in the antiviral activity. A number of recent studies have suggested that an element crucial for the chemokine-induced receptor activation is located at the molecule's NH_2-terminal (J. Biol. Chem. 1991; 266:23128-23134; Biochem. Biophys. Comm. 1995, 211:100-105), which does not apparently possess a specific structure in solution, as determined by means of NMR analysis, and which thus seems "mobile" (Biochemistry 34:5329-5342:1995). Actually, a preliminary study (Nature, 1996, 383:400) and a more detailed study (Science; 1997, 276-282), both recently published, have shown that RANTES-chemokine analogues modified at the NH_2 terminal (through the deletion of 8 amino acids, or through the covalent bond of a complex chemical radical [amino-oxy-pentane or AOP], respectively) maintain the anti-HIV activity even though they do not induce chemotaxis in vitro or they induce it at a very low extent.

Such results confirm that the pro-inflammatory and antiviral activities of RANTES can be dissociated and that the same activities presumably depend on two or more distinct protein domains.

Summary of the invention

Now it has been found that a RANTES domain or fragment, homologous to the colinear MIP-1α and MIP-1β segments, can block the HIV infection. The such antiviral domain will be hereafter referred to as π1.

According to a first aspect, the invention provides peptides having from 5 to 30 amino acids, with a sequence corresponding or homologous to the sequence 5-34 of RANTES or to the sequence 6-35 of MIP-1α and MIP-1β or to the colinear sequences of different chemokines binding the CCR5 receptor. The sequence 5-34 of RANTES and the sequence 6-35 of MIP-1α and MIP-1β comprise the sequence between the second and the third cysteine which is considered essential for binding viral coreceptors.

Further, the invention is directed to peptides from the region between the second and the third cysteine of SDF-1 chemokine or of other chemokines which bind $CXCR_4$, as well as of other chemokines which bind those receptors or other receptors used by HIV-1 virus as membrane coreceptors from the target cell.

By homologous sequence it is intended an amino acid sequence having at least 60%, preferably at least 80% and more preferably at least 90% homology with the sequence comprised between the positions 5 and 34 of RANTES or 6-35 of MIP-1α or MIP-1β.

According to one aspect, the invention provides derivatives of said peptides, chemically modified in

order to increase their in vivo stability.

According to a further aspect, the invention provides chimeric proteins which are obtained through conventional techniques by inserting the antiviral domain described above, into proteins showing the desired biological characteristics.

Finally, the invention provides antiviral, antiinflammatory and antiallergic pharmaceutical compositions containing the above defined peptides or proteins as the active ingredient.

Preferably, the peptides of the invention are represented by the following formula (I):

$$XCFX_1YX_2X_3RX_4 \qquad (I)$$

wherein C, F, Y and R respectively correspond to cysteine, phenilalanine, thyrosine and arginine according to amino acid one-letter code;

X is the cysteine NH_2-terminal, cysteine or a peptide comprising from 2 to 5 amino acids of the sequence (reported in Fig.1) corresponding to the positions 6-10 of RANTES, MIP-1α, MIP-1β;

X_1 is alanine or serine (A or S);

X_2 is isoleucine or threonine (I or T);

X_3 is alanine or serine (A or S);

X_4 is proline, lysine or glutamine (P, K or Q) or it is a peptidic sequence comprising from 2 to 18 amino acids wherein the first amino acid is proline, lysine or glutamine and the other amino acids correspond to the sequence 19-35 of RANTES, MIP-1α, MIP-1β, reported in Fig. 1.

Preferably, the peptides of the invention comprise from 8 to 20 amino acids, more preferably from 8 to 16

amino acids and even more preferably from 8 to 12 amino acids.

According to formula (I), X is preferably the NH_2 terminal of cysteine and X_4 is preferably selected from proline, glutamine and lysine or it is a peptide with a sequence selected from PL, PLP, PLPR, PLPRA, QI, QIP, QIPQ, KL, KLP, KLPR.

The following peptides are examples of peptides of formula (I):

1) TTPCCFAYIARP (Sequence Id N. 1)
2) PCCFAYIARPLP (Sequence Id N. 2)
3) CFAYIARPLPRA (Sequence Id N. 3)
4) CCFSYTSRQIPQ (Sequence Id N. 4)
5) PPTACCFSYTAT (Sequence Id N. 5)
6) TACCFSYTARKL (Sequence Id N. 6)
7) CCFSYTARKLPR (Sequence Id N. 7)
8) TPTACCFSYTSR (Sequence Id N. 8)
9) TACCFSYTSRQI (Sequence Id N. 9)

The CFAYIARP sequence is particularly preferred. Such sequences, also the partial sequences, or the sequences which differ for the substitution of at most two amino acids with other amino acids of the same kind (neutral, acidic or basic, hydrophobic or hydrophilic, big- or small-sized amino acids), can be inserted in or linked to sequences of physiologic proteins which serve as non-toxic carriers for the π1 antiviral domain or, more precisely, for the HIV-suppressive domain.

Human albumin or the Fc γ fragment of IgG human immunoglobulin are examples of physiologic proteins.

Further, the invention comprises derivatives and analogues of the peptides of formula (I), having non-

natural D-amino acids, retro inverted bonds or functions on the $-NH_2-$ or -COOH terminals or on the COOH, OH, SH and NH_2 groups, in order to increase the metabolic resistance of the peptide without affecting the antiviral properties. Further, the invention comprises peptides having the sequence of formula (I) which is repeated from 2 to 10 times ("tandem repeats"), and cyclic peptides.

The invention comprises also peptides with at least 60% homology, preferably at least 80% homology with the sequences comprised in formula (I).

Some amino acid substitutions are known to maintain the biologic activity of the corresponding peptides. See for example H. Neurath and R.L. Hill "The proteins", Academic Press, N.Y. (1979). Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Gly, Asp/Gly, and the reciprocal, are the more frequent substitutions which do not normally affect the biologic activity.

Description of the Figures

Figure 1 shows the amino acid sequences of the human C-C chemokines RANTES, MIP-1α, MIP-1β.

Figure 2 shows the T-limphocyte binding of peptides having 15 amino acids, 12 of which are derived from the complete sequence of RANTES, which are sequentially overlapped, spaced from one another by two amino acids, the other three amino acids (G-S-G) being added to the COOH- terminal of each peptide for the biotinylation. The different peptides are indicated on the axis of abscissas, according to the initial residue which

occupies the first position of the mature peptide (after removal of the signal peptide);

Figure 3 shows the inhibition of the HIV-1 induced syncytium in limphoid T-cells by the same peptides of Figure 2.

Detailed description of the invention

The peptides of the invention are prepared with the conventional methods of peptide synthesis, in solid and homogeneous phases.

A typical solid-phase procedure comprises:

a) condensation of an amino acid protected at the α-amino group, with an insoluble solid support, through the formation of an amide between the amino acid carboxyl and the amino group of the solid support;

b) removal of the protecting α-amino group;

c) condensation of the amino acid bound to the insoluble support with a further amino acid protected at the α-amino group, through the reaction between the de-protected amino group of the first amino acid and the activated carboxyl of the second amino acid;

d) removal of the protecting α-amino group of the second amino acid;

e) addition of other amino acids, in consecutive steps, according to the steps b-d, until the desired peptidic chain is completed;

f) removal of the synthesized peptide from the insoluble solid support and the purification thereof.

For the synthesis of the peptides of formula (I),

polystyrenic resins cross linked by about 1%-2% divinylbenzene and functionalized with a spacer of polyethylenglycole, are preferably used as the solid support.

The C-terminal amino acid suitably protected at the amino group, is condensed with the activated resin through the formation of an ester or amide between the carboxylic group and the linker present on the resin.

9-fluorenylmethyloxycarbonyl (Fmoc) is preferred among the α-amino-protecting groups since it can be easily removed in mild conditions.

The removal of said protecting group is carried out with 20% pyperidine basic solution in dimethylformamide.

The reactive functional groups present in the amino acid side chains are generally protected with the protecting groups known in the peptide synthesis, which keep stable under the conditions used for the removal of the α-amino protecting group.

In the process steps c) and e) the amino acids are added to the growing peptidic chain in form of active esters, using dimethylformamide or methylene chloride as inert solvents.

At the end of the synthesis, in step f) the peptide is removed from the solid support by means of acidic hydrolysis. Then, the resin-peptide is suspended in a solution of trichloroacetic acid containing 10-12% of scavengers like thioanisole, water, phenol, trithylsilane, ethandithiole. The suspension is maintained at room temperature for three hours, then the resin is separated and the solution is concentrated. The peptide is precipitated and extensively washed with

ethylic ether. The so obtained peptide is dissolved in acidic aqueous solvent and then purified with chromatography. Subsequently, the fractions containing the peptide are collected and lyophilized.

According to this technique, a number of peptides containing 15 amino acids have been synthesized. 12 of those amino acids are derived from the complete sequence of RANTES, and they are sequentially overlapped, spaced from one another by two amino acids, while the other three (Gly-Ser-Gly) are added to each peptide at the COOH-terminal to allow the biotinylation of the peptide, in order to assess their human cell binding.

For this purpose, the $CD4^+$PM1 T-limphoid cell line has been used (Lusso et al.., J. Virology, 1995, 69:3712-3720).

It has been found that the peptide/T-limphocyte binding does not involve a single region of the sequence, but it is a common property shared by the peptides from at least three distant regions which are separated in the complete sequence.

On the contrary, it has surprisingly come out that the antiviral activity is exhibited by peptides from a single region, having a sequence comprised between the second and the third cysteine of the C-C chemokine sequence, as defined above.

The inhibition of the syncitium (i.e. of the giant multinuclear cell produced by the fusion of infected cells with non-infected cells) was used as the antiviral activity test, after 18 hr incubation of non-infected PM1 cells with PM1 cells stably infected by the viral strain HIV-BaL (which uses the CCR5 coreceptor).

The results obtained with some peptides of the invention are reported in Figure 3.

The peptides of the invention did not exert any activity when the same test was carried out with the HIV-1 strain IIIB, adapted to the growth in continuous cell lines, which strain displays a different membrane coreceptor ($CXCR_4$) and thus it does not respond to the above mentioned chemokine. The same results were obtained in respect of HIV-1 Bal and HIV-1 IIIB also using dodecapeptides having the same sequence of the peptides listed above, but lacking the GSG tripeptide used for the biotinylation.

The peptides of the invention, the derivatives thereof, or the chimeric proteins in which they are contained, can be used for the therapy or the prophylaxis of AIDS and of other diseases which are caused by the infection of primate lentiretrovirus and of other viruses which bind the chemokine receptors. Further, the peptides of the invention can be used for the treatment of allergic or autoimmune diseases, or for the treatment of any other disease in the pathogenesis of which the chemokines are involved. The peptides of the invention will be administered suitably formulated in pharmaceutical compositions, for example as reported in "Remington's Pharmaceutical Sciences Handbook", Mack Publishing Company, New York, U.S.A..

The compositions of the invention will contain an effective amount of the peptides (or the derivatives thereof and the chimeric proteins), for instance from 0.1 to 100 mg of peptide, and they will be administered preferably by the parenteral route, in particular by the

subcutaneous or intramuscular routes. The daily amount will obviously depend on different factors, like severity of the disease, weight, sex and age of the patient, and it will be determined on the basis of the toxicological, pharmacokinetic and pharmacodynamic properties of each single peptide or derivative thereof.

Generally the peptide daily dosage will be comprised between 10 and 1500 µmol per Kg of body weight and the treatment will be maintained for a long time. Also other administration routes can be used, for example the oral route using liposome formulations or other techniques known for the administration of peptides or proteins by the gastroenteric route, as described in WO93/25583.

Further, the peptides of the invention can be used for the production of anti-peptide antibodies and anti-idiotype antibodies raised to the anti-peptide antibody, which anti-idiotype antibodies simulate the original peptide through their active site. Moreover, the peptides can be used for the development of peptide mimetics with antiviral activity or for the development of antagonists of the chemokine receptor.

Such antibodies, optionally human antibodies, have a favourable diffusion and stability and a longer half life in vivo.

The techniques used for the production of anti-idiotype antibodies and human antibodies are described for example in WO 86/1539 and in EP-A-481790.

The peptides of the invention are useful also as diagnostic and research tools, for instance for the structural characterization of the active site by means

of computer aided-molecular design, crystallography or NMR.

The following examples illustrate the invention in greater details.

EXAMPLE 1

The biotinylated peptides newly synthesized were dissolved in pure DMSO and then diluted in phosphate buffer (PBS) to obtain a 66% stock of 1.66 mg/ml in DMSO.

For the cellular surface binding test, the biotin-coniugated peptides were incubated at a concentration of 100 ug/ml in a volume of 100 µl PBS with 100,000 cells of the PM1 line, which were previously washed twice with PBS. The PM1 line, incubated with a 66%-NDMSO water solution, was used in the control test. After 30 min at a temperature of 4 °C, the cells were washed two times with PBS and incubated in 100 µl of PBS together with FITC fluochrome-coniugated avidin (Sigma) at a concentration of 5 µg/ml. After 30 min at 4°C, the cells were washed again with PBS and resuspended in 500 µl PBS for the cytofluorimeter reading (Becton Dickinson). 10,000 events were collected for each sample. The following values were attributed to the cell surface binding: no binding (fluorescence profile overlapping with the negative control)= - (0); fluorescence peak lower than 20=+/- (19); fluorescence peak between 20 and 100=+(30); fluorescence peak between 100 and 500=++(60); fluorescence peak higher than 500=+++ (90). The results are reported in Fig. 2.

EXAMPLE 2

Two types of synthetic peptides were used for the

HIV-induced syncitium-inhibition test: the biotin conjugated peptides described above (15mer) and some peptides of identical sequence without biotin and tail (GSG (12mer) (Neosystem), firstly dissolved in water at a concentration of 5 mg/ml and then in PBS at a stock concentration of 1 mg/ml. Two couples of continuous cell lines were used: 1) the non-infected PM1 cell line and the same cell line chronically infected by the HIV-1Bal virus; 2) the non infected Molt-3 cell line and the same cell line chronically infected by the HIV-1IIIB virus. All the cell lines rapidly originate the multinuclear giant cells, or syncitia, when they are brought into contact in the same colture, thanks to the spindle-generating property of the virus envelope proteins present in the infected cell line. The presence of the complete receptor for the HIV-1 virus on the non-infected cells is an essential condition for generating a syncitium. At the beginning the cells from the four cell lines were repeatedly washed with PBS. Then, 100,000 cells of the non-infected cell line (PM1 or Molt-3) were seeded in 96- well flat bottomed plates (Costar) and incubated for 30 min at room temperature and a final concentration of 10 ug/ml for each peptide in a final volume of 200 µl. After 18 hr, the cells were microscopically examined. Two different operators were assigned separately to the count of the syncitia, according to a blind protocol. In the graph, the following values were attributed to the syncitium inhibition: no syncitium= -(90); few syncitia of small size= +/- (60); many syncitia of medium size= ++(10); a great number of syncitia of big size=+++ (0). The

results are reported in Fig. 3.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: PEPTIDES WITH ANTIVIRAL ACTIVITY

(iii) NUMBER OF SEQUENCES: 9

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: IT

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Thr Thr Pro Cys Cys Phe Ala Tyr Ile Ala Arg Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Pro Cys Cys Phe Ala Tyr Ile Ala Arg Pro Leu Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Cys Phe Ala Tyr Ile Ala Arg Pro Leu Pro Arg Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Pro Pro Thr Ala Cys Cys Phe Ser Tyr Thr Ala Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Thr Ala Cys Cys Phe Ser Tyr Thr Ala Arg Lys Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Cys Cys Phe Ser Tyr Thr Ala Arg Lys Leu Pro Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Thr Pro Thr Ala Cys Cys Phe Ser Tyr Thr Ser Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Thr Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile
1 5 10

CLAIMS

1. Peptides having from 5 to 30 aminoacids, with a sequence homologous or corresponding to the sequence 5-34 of RANTES or 6-35 of MIP-1α, MIP-1β or to the colinear sequences of other chemokines which bind the CCR5 receptor.

2. Peptides having from 5 to 30 amino acids with a sequence homologous or corresponding to the sequence comprised between the second and the third cysteine of SDF-1 or of other chemokines binding $CXCR_4$.

3. Peptides according to claim 1 having from 5 to 20 amino acids.

4. Peptides according to claim 1 having from 8 to 12 amino acids.

5. Peptides according to any of the claims 1-3 with a sequence corresponding to the sequence comprised between the second and the third cysteine of RANTES.

6. Peptides according to any of the claims 1-4 having the sequence

$$XCFX_1YX_2X_3RX_4 \qquad (I)$$

wherein C, F, Y, and R are cysteine, phenylalanine, thyrosine, and arginine, respectively, according to the amino acid one-letter code;

X is the NH_2 terminal of cysteine or cysteine or the peptide comprising from 2 to 5 amino acids corresponding to the positions 6-10 of RANTES, MIP-1α and MIP-1β, as reported in Fig. 1;

X_1 is alanine or serine (A or S);

X_2 is isoleucine or threonine (I or T);

X_3 is alanine or serine (A or S);

X_4 is proline, lysine, or glutamine (P, K, or Q) or a sequence comprising from 2 to 18 amino acids wherein the first amino acid is proline, lysine or glutamine and the other amino acids correspond to the sequence 19-35 of RANTES, MIP-1α and MIP-1β.

7. Peptides according to claim 5 wherein X is the cysteine NH_2-terminal and X_4 is selected from proline, glutamine and lysine or it is a peptide having a sequence selected from PL, PLP, PLPR, PLPRA, QI, QIP, QIPQ, KL, KLP, KLPR.

8. Peptides according to claim 6 having a sequence selected from the group consisting of:

1) TTPCCFAYIARP (Sequence Id N. 1)
2) PCCFAYIARPLP (Sequence Id N. 2)
3) CFAYIARPLPRA (Sequence Id N. 3)
4) CCFSYTSRQIPQ (Sequence Id N. 4)
5) PPTACCFSYTAT (Sequence Id N. 5)
6) TACCFSYTARKL (Sequence Id N. 6)
7) CCFSYTARKLPR (Sequence Id N. 7)
8) TPTACCFSYTSR (Sequence Id N. 8)
9) TACCFSYTSRQI (Sequence Id N. 9)

9. Chimeric proteins obtainable by inserting the peptides of claims 1-8 into physiologic proteins.

10. Proteins according to claim 9, wherein the physiologic proteins are selected from albumin or immunoglobulin G-Fc fragment.

11. Analogues of the peptides of claims 1-9 wherein one or more amino acids are D-amino acids or they have been functionalized on NH_2- and COOH- terminals or on COOH, NH_2, OH and SH with groups which increase the metabolic stability of the peptides.

12. Peptides having at least 60% homology with the peptides of claim 5.

13. Pharmaceutic compositions containing the peptides of claims 1-12 as the active ingredient.

14. Use of the peptides of claims 1-12 for the preparation of medicaments with antilentiretrovirus activity.

15. Use of the peptides according to claim 14 wherein the lentiretrovirus is HIV-1.

16. Use of the peptides of claims 1-12 for the preparation of medicaments with antiallergic and antiinflammatory activity.

17. Antibodies raised to the active site of antibodies which bind the peptides of claims 1-12.

SHEET 3/3

Inhibition of the HIV-1-induced syncitium by RANTES peptides

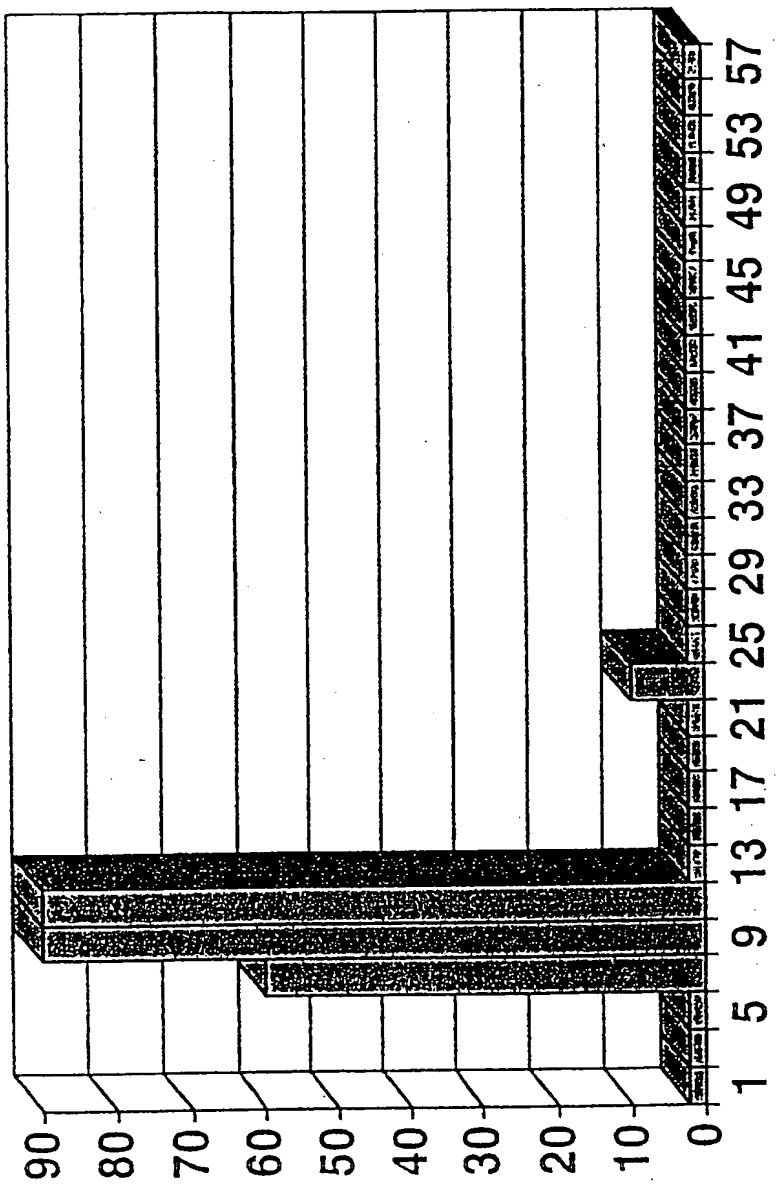

FIGURE 3

SHEET 2/3

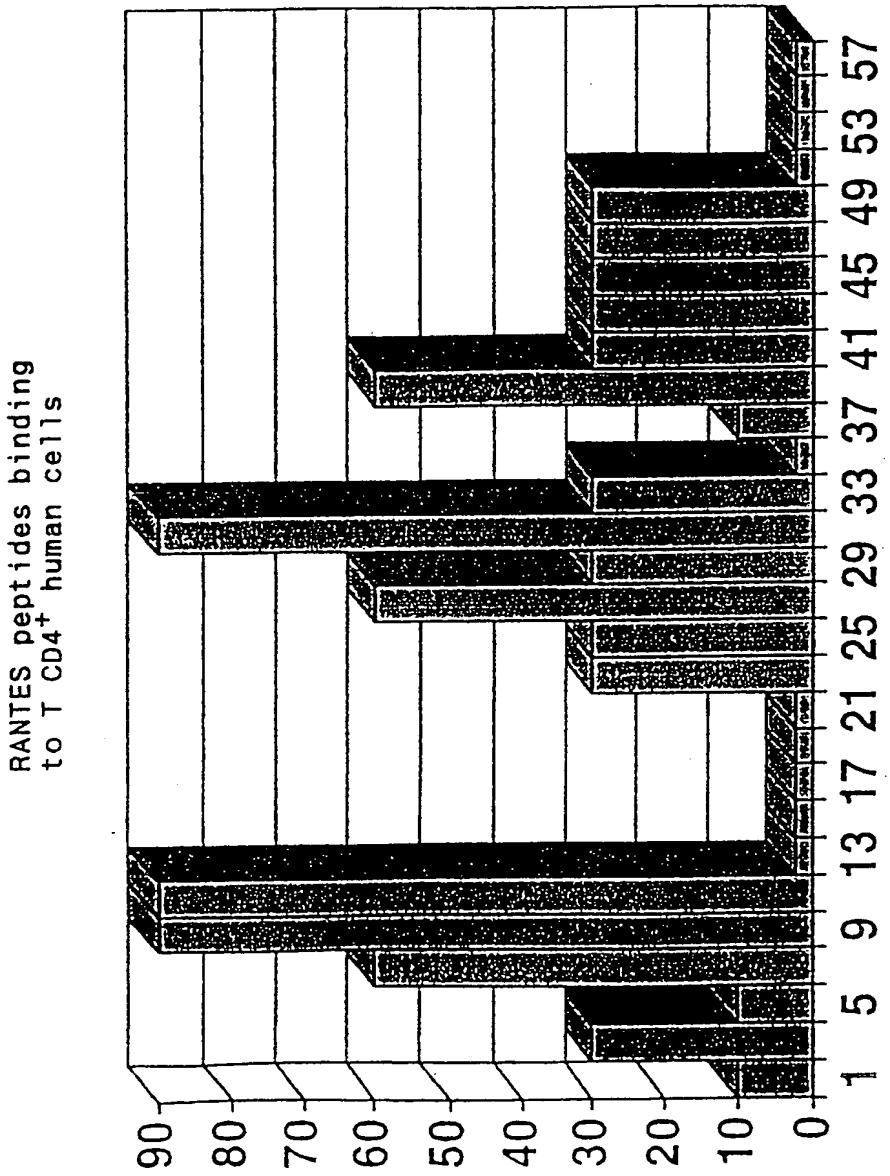

FIGURE 2

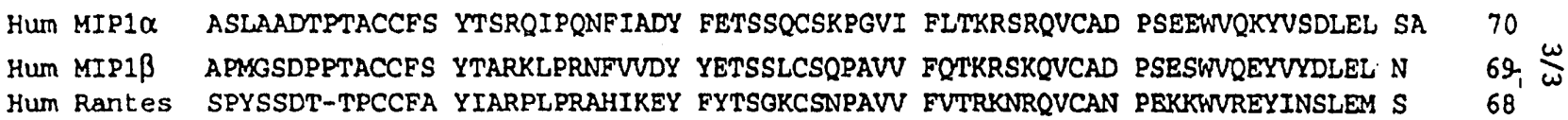
Hum MIP1α ASLAADTPTACCFS YTSRQIPQNFIADY FETSSQCSKPGVI FLTKRSRQVCAD PSEEWVQKYVSDLEL SA 70
Hum MIP1β APMGSDPPTACCFS YTARKLPRNFVVDY YETSSLCSQPAVV FQTKRSKQVCAD PSESWVQEYVYDLEL N 69
Hum Rantes SPYSSDT-TPCCFA YIARPLPRAHIKEY FYTSGKCSNPAVV FVTRKNRQVCAN PEKKWVREYINSLEM S 68

FIGURE 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 98/02750

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/52, A61K 38/19, C07K 16/24
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 7067689 A (ZH KAGAKU & KESSEI RYOHO KENKYUSHO), 14 March 1995 (14.03.95), page 8, line 18 - line 22, & WPI abstract, Derwent accession no. 95-143871	1-12,17
A	--	13-16
X	Nature, Volume 383, October 1996, Fernando Arenzana-Seisdedos et al, "HIV blocked by chemokine antagonist"	1-17
	--	
X	WO 9617935 A2 (GLAXO GROUP LIMITED), 13 June 1996 (13.06.96), see page 5, line 30 - page 6, line 6	1-13,16-17
A	--	14-15

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 Sept 1998	05 OCT 1998
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Authorized officer PATRICK ANDERSSON

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 98/02750

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Science, Volume 270, December 1995, Fiorenza Cocchi et al, "Identification of RANTES, MIP-1alpha, and MIP-1beta as the Major HIV-Suppressive Factors Produced by CD8+ T Cells" page 1811 - page 1815	1-17
A	Dialog Information Services, File 34, SciSearch, Dialog accession no. 05309415, Rucker J. et al: "Regions In Beta-Chemokine Receptors CCR5 And CCR2B That Determine HIV-1 Cofactor Specificity", Cell, 1996, V87, N3 (NOV 1), p 437-446	1-17
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P,X	WO 9806751 A1 (RESEARCH CORPORATION TECHNOLOGIES, INC.), 19 February 1998 (19.02.98)	1-17
P,X	WO 9813495 A1 (BRITISH BIOTECH PHARMACEUTICALS LIMITED), 2 April 1998 (02.04.98)	1-17

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Information on patent family members

International application No.
PCT/EP 98/02750

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WO 9813495 A1	02/04/98	AU 4561497 A	17/04/98